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Identification of Cell Types Responsible for Bone Resorption in Rheumatoid Arthritis and Juvenile Rheumatoid Arthritis

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Focal resorption of bone at the bone-pannus interface is common in rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA) and can result in significant morbidity. However, the specific cellular and hormonal mechanisms involved in this process are not well established. We examined tissue sections from areas of bone erosion in patients with RA and JRA. Multinucleated cells (MNCs) were present in resorption lacunae in areas of calcified cartilage and in subchondral bone immediately adjacent to calcified cartilage, as previously described. mRNA for the calcitonin receptor (CTR) was localized to these MNCs in bone resorption lacunae, a finding that definitively identifies these cells as osteoclasts. These MNCs were also positive for tartrate-resistant acid phosphatase (TRAP) mRNA and TRAP enzymatic activity. Occasional mononuclear cells on the bone surface were also CTR positive. Mononuclear cells and MNCs not on bone surfaces were CTR negative. The restriction of CTR-positive cells to the surface of mineralized tissues suggests that bone and/or calcified cartilage provide signals that are critical for the differentiation of hematopoietic osteoclast precursors to fully differentiated osteoclasts. Some MNCs and mononuclear cells off bone and within invading tissues were TRAP positive. These cells likely represent the precursors of the CTR-TRAP-positive cells on bone. Parathyroid hormone receptor mRNA was present in cells with the phenotypic appearance of osteoblasts, in close proximity to MNCs, and in occasional cells within pannus tissue, but not in the MNCs in bone resorption lacunae. These findings demonstrate that osteoclasts within the rheumatoid lesion do not express parathyroid hormone receptor. In conclusion, the resorbing cells in RA exhibit a definitive osteoclastic phenotype, suggesting that pharmacological agents that inhibit osteoclast recruitment or activity are rational targets for blocking focal bone erosion in patients with RA and JRA. *(Am J Pathol 1998, 152:943–951)*

Rheumatoid arthritis (RA) and juvenile rheumatoid arthritis (JRA) are systemic rheumatic diseases in which bone loss is a major cause of morbidity. Bone loss in RA has been extensively reviewed in the clinical literature, with the conclusion that three distinct forms of bone loss occur: generalized osteoporosis distal from diarthrodial joints, periarticular osteopenia adjacent to inflamed joints, and erosion of marginal and subchondral bone at the bone-pannus interface. Analysis of bone biopsies from patients with RA by histomorphometry demonstrates that generalized osteoporosis is due to thinning of trabecular bone.[1] Additional studies suggest that this loss of trabecular bone is due, at least in part, to a decrease in bone formation rather than an increase in bone resorption.[2,3] More recently, however, studies with biochemical markers of bone turnover have suggested a generalized increase in the rate of bone resorption in patients with RA[4,5] and in patients with RA using low-dose corticosteroids.[6]

Periarticular osteopenia usually occurs in joints with active synovial inflammation. Although the exact pathogenesis of this form of bone loss is not clear, histomorphometric analysis of bone specimens from patients with RA have concluded that there is an increase in juxtaarticular bone turnover and in particular in bone resorption in RA samples in comparison with samples from patients with osteoarthritis.[7] Joint immobility, increased

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synovial vascularity, and elaboration of mediators of inflammation by synovial tissues have all been implicated as mechanisms to explain this form of bone loss.[8–11]

The final form of bone loss, erosion of marginal and subchondral bone in diarthrodial joints, is a typical feature of RA and JRA. At the interface between synovium and bone, hyperplastic synovial tissue extends over the surface of the articular cartilage, forming pannus that invades and destroys cartilage. In areas of pannus infiltration, erosion of calcified cartilage and subchondral bone is common, leading to characteristic marginal erosions seen radiographically in these diseases. Although the role of enzymes, including matrix metalloproteinases,[12] cathepsins[13] and mast cell proteases,[14] in the degradation of cartilage at this site have been elucidated, the specific cellular mechanisms underlying marginal and subchondral bone loss are not well established.

The role of osteoclasts in erosion of bone in RA has been suggested by several studies. Bromley and Woolley[15] analyzed tissue from the bone-cartilage junction in samples from RA patients obtained at the time of joint replacement surgery. Acid-phosphatase-positive multinucleated cells (MNCs) with phenotypic features of osteoclasts were noted in areas of subchondral bone loss and were directly associated with mineralized subchondral bone or with mineralized and unmineralized cartilage. These cells were designated by histological appearance and location as osteoclasts or chondroclasts, the latter being MNCs located on mineralized or unmineralized cartilage. Subchondral tissue damage extended into unmineralized cartilage in approximately 30% of samples studied. At the time of these studies, however, no definitive markers of osteoclasts were available. Electron microscopic studies on the topography of rheumatoid metacarpal heads in areas of pannus invasion have noted resorption bays typical of osteoclastic activity over the surfaces of calcified cartilage and subchondral bone.[16] These studies demonstrated that the area of contact between pannus and cartilage/bone represented the erosive front. In addition, characterization of cells isolated from rheumatoid synovium suggest that cells with phenotypic features of osteoclasts are involved in marginal bone erosion in RA.[17,18]

Studies such as these have been hindered by the absence of a unique, definitive marker to distinguish osteoclasts from the other cells of the monocyte/macrophage lineage. Expression of tartrate-resistant acid phosphatase (TRAP) activity is a characteristic phenotypic marker of osteoclasts and is expressed in osteoclasts resorbing bone. Although used as a standard marker of osteoclasts, TRAP activity can be present in other cells, including macrophage polykaryons generated in murine bone marrow culture[19,20] as well as in human bone marrow macrophages in pathological states.[21] The cloning of the calcitonin receptor (CTR)[22,23] has provided important reagents for the definitive identification of osteoclasts in pathological tissue sections. The expression of the CTR has been demonstrated to be a definitive marker that distinguishes osteoclasts from other hematopoietic and bone cells.[20,24–28] *In vitro* studies have established that expression of CTR occurs in mononuclear cells before fusion to form osteoclasts, and its expression is also associated with competence to resorb bone.[24–28] We studied samples from the cartilage and bone-pannus interface from patients with RA and JRA to elucidate the cellular basis of erosion of marginal and subchondral bone in these diseases.

Materials and Methods

Tissue Procurement

Tissue samples from the pannus-bone and pannus-cartilage interface were obtained as discarded material from joint replacement surgery in three patients with RA and one patient with polyarticular JRA. One of four patients was taking prednisone at the time of joint replacement surgery at a dose of 10 mg po qd. Joints examined included one elbow and three knees. Tissue procurement was approved by the Internal Review Board. Samples were fixed in 4% paraformaldehyde or 10% neutral buffered formalin for 2 days, followed by decalcification in 14% EDTA for up to 5 weeks or in decalcifying solution F (Baxter Pharmaceuticals, McGaw Park, IL) for 3 days. Decalcified samples were either paraffin embedded or immediately frozen in optimal temperature compound (OCT, Miles Laboratory, Elkhart, IN) immersed in 2-methyl butane in a liquid nitrogen bath. Frozen tissues were stored at −80°C.

In Situ *Hybridization*

Tissue Preparation

Fixed and decalcified synovial tissues for paraffin embedding were washed in graded ethanol solutions and stored overnight at 4°C in 100% ethanol. Samples were transferred to xylenes and then embedded in paraffin. Paraffin sections were placed on 3-aminopropyltriethoxysilane-coated slides, dried overnight, and used immediately or stored at 4°C.

Probe Preparation

Sense and antisense ^{35}S-labeled CTR cRNA probes were prepared from a 642-bp fragment of the human CTR cDNA corresponding to the region of the receptor extending from the predicted second transmembrane-spanning domain to the proximal portion of the fourth intracellular domain, subcloned into pSK Bluescript (Stratagene, La Jolla, CA). Sense and antisense ^{35}S-labeled probes for TRAP were prepared from a 382-bp fragment of the human TRAP cDNA generated by polymerase chain reaction using cDNA from a human osteoclast library subcloned into pCRII, prepared by Dr. David Roodman, University of Texas, San Antonio, TX. A 741-bp fragment of the human parathyroid hormone receptor (PTHR) was generated from the human osteosarcoma cell line SAOS-2 by Dr. Harold Jueppner, Massachusetts General Hospital, Boston, MA, by reverse transcriptase polymerase chain reaction and subcloned

into pcDNA1. The probe includes the region from the first extracellular domain to the second transmembrane region. Vectors were appropriately linearized and incubated with either T7 or T3 RNA polymerase in the presence of ^{35}S-labeled UTP (New England Nuclear, Boston, MA), unlabeled nucleotides, 10 mmol/L dithiothreitol, and RNAse inhibitor (Promega, Madison, WI). Labeled RNA probes were separated from free nucleotides using NucTrap push columns (Stratagene, La Jolla, CA).

Prehybridization

Slides were deparaffinized in xylenes followed by rehydration in graded ethanol solutions, rinsed in 0.85% NaCl for 5 minutes and 1X PBS for 5 minutes. Snap-frozen tissue sections were used directly. Sections were re-fixed in 4% paraformaldehyde for 20 minutes, washed in 1X PBS, and treated with proteinase K (20 μg/ml) for 15 to 20 minutes. Slides were dipped successively in 1X PBS (5 minutes) and 4% paraformaldehyde (5 minutes), acetylated in 0.25% acetic anhydride in 100 mmol/L triethanolamine, washed in 1X PBS (5 minutes) and 0.85% NaCl (5 minutes), dehydrated in graded ethanol solutions, and air dried.

Hybridization

Hybridization solution contained 2X SSC, 50% formamide, 10 mmol/L Tris/HCl (pH 7.5), 1 mmol/L EDTA (pH 8.0), 10% dextran sulfate, 1X Denhardt's solution, and 0.5 mg/ml total yeast RNA plus 50 μg/ml yeast tRNA, 100 μg/ml denatured salmon sperm DNA, 100 mmol/L dithiothreitol, 0.1% SDS, and 0.1% sodium thiosulfate. cRNA probes labeled with ^{35}S were heated at 80°C for 2 minutes and placed on ice. Hybridization solution containing probe (2×10^4 cpm/μl) was added to each slide in a 120-μl volume, a glass coverslip was placed on each slide, and slides were incubated at 55°C for 16 hours. After hybridization, slides were washed four times at room temperature in 2X SSC, 10 mmol/L 2-mercaptoethanol, and 1 mmol/L EDTA for 5 minutes each. Slides were then treated with a 20 μg/ml solution of RNAse A (Pharmacia Biotech, Piscataway, NJ) for 30 minutes, washed at 60°C in 0.1X SSC, 10 mmol/L 2-mercaptoethanol, and 1 mmol/L EDTA for 2 hours, rinsed in 0.5X SSC three times at room temperature for 5 minutes each, dehydrated in graded ammonium acetate/ethanol solutions, and air dried.

Autoradiography

Air-dried slides were dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY), drained and air dried for 1 hour, and placed in a light-proof container with dessicant at 4°C for 10 to 14 weeks. Slides were developed in Kodak D-19 developer, fixed in Kodak fixer, and counterstained with hematoxylin and eosin (H&E).

Immunohistochemistry

Four-micron paraffin-embedded sections were placed on 3-aminopropyltriethoxysilane-coated glass microscope slides. Primary antibody was anti-CD68 (KP1, Dako, Carpinteria, CA) used at a dilution of 1:40. Slides were deparaffinized in xylenes, hydrated in graded ethanol solutions, and placed in 0.05 mol/L Tris for three washes of 5 minutes each. Slides were lightly trypsinized before blocking in 20% goat serum for 20 minutes. Slides were incubated for 1 hour with the primary antibody, washed three times for 5 minutes each in 0.05 mol/L Tris, pH 7.5, and incubated with biotin-conjugated goat anti-mouse IgG diluted 1:200 (Southern Biotechnology, Birmingham, AL), washed three times for 5 minutes each, and incubated for 1 hour with streptavidin-conjugated alkaline phosphatase diluted 1:500 (Boehringer Mannheim, Indianapolis, IN). Slides were washed three times for 5 minutes each followed by color development with PhThalo Red solution (Kirkegaard and Perry, Gaithersburg, MD) and counterstained with hematoxylin before the application of Crystal/mount (Biomeda, Foster City, CA). Negative control antibody was MOPC-21 (Sigma Chemical Co., St. Louis, MO), used at the same concentration as the KP-1 antibody.

TRAP Staining

TRAP staining was performed by a modification of a previously published method.[29] Briefly, 4- to 6-μm tissue sections were run in parallel for acid phosphatase alone or with 25 mmol/L sodium tartrate. Sections were incubated at 37°C for 40 minutes in freshly prepared 0.1 mol/L Tris buffer, pH 5.0, containing 1.35 mmol/L naphthol AS-MX phosphate (Sigma), 0.362 mol/L *N,N*-dimethylformamide, 3.88 mmol/L Fast Red TR salt, 0.5 mmol/L manganese chloride, and 25 mmol/L sodium tartrate. Slides were rinsed for 10 minutes and counterstained with hematoxylin.

Results

We examined three cases of RA and one case of polyarticular JRA to identify bone-resorbing cells and their precursors at the bone-pannus and cartilage-pannus interface. As has been previously noted,[15] MNCs in RA are located in resorption lacunae in areas of calcified cartilage and in subchondral bone just deep to areas of calcified cartilage.

Expression of TRAP activity is a phenotypic marker that has been used to identify osteoclasts and osteoclast precursors. TRAP staining was performed on paraffin-embedded tissue sections from the bone-pannus and cartilage-pannus interface. MNCs in resorption lacunae on the bone surface were uniformly TRAP positive (Figure 1). Many mononuclear cells on the bone surface were also TRAP positive, suggesting that these cells may be osteoclast precursors that have up-regulated TRAP enzyme activity before fusion. In addition, several mononuclear cells and MNCs located within pannus in areas

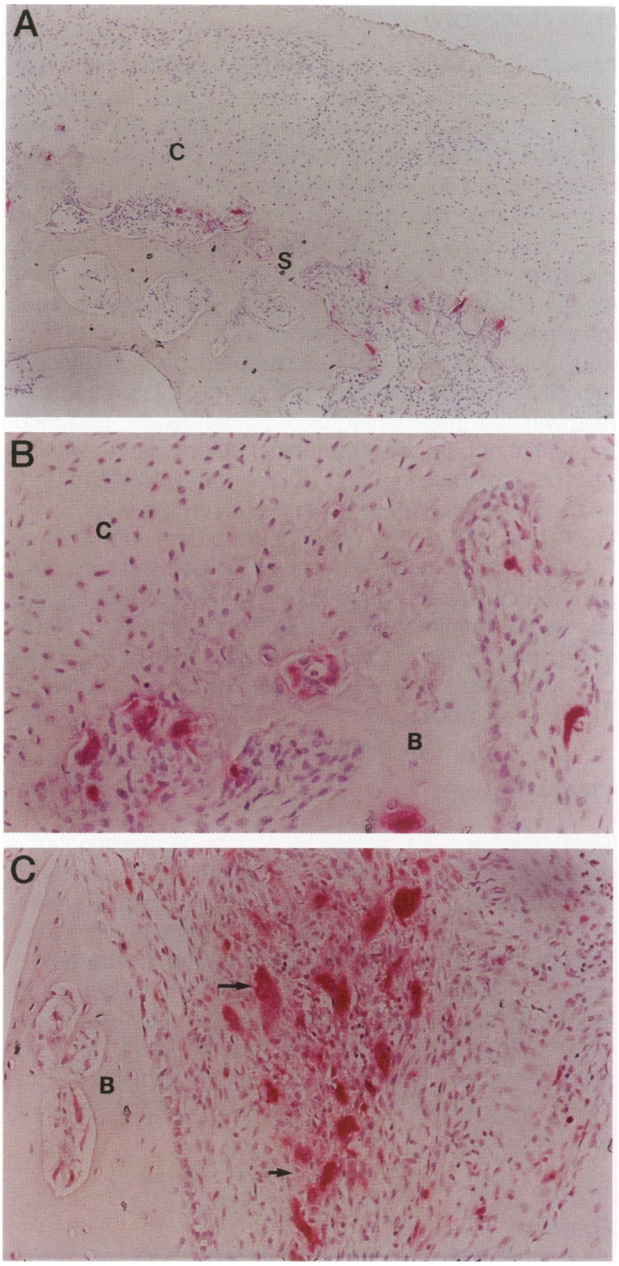

Figure 1. TRAP staining in joint tissue from a patient with polyarticular juvenile rheumatoid arthritis (JRA). **A:** Low-power view demonstrating TRAP-positive MNCs in bone resorption lacunae just deep to fibrous pannus and cartilage at the interface between calcified cartilage and subchondral bone. S, subchondral bone; C, cartilage. Hematoxylin counterstain; magnification, ×20. **B:** TRAP-positive MNCs on bone. B, bone; C, cartilage. Hematoxylin counterstain; magnification, ×66. **C:** Numerous TRAP-positive MNCs (**long arrow**) and mononuclear cells (**short arrow**) in pannus remote from bone. B, bone. Hematoxylin counterstain; magnification, ×50.

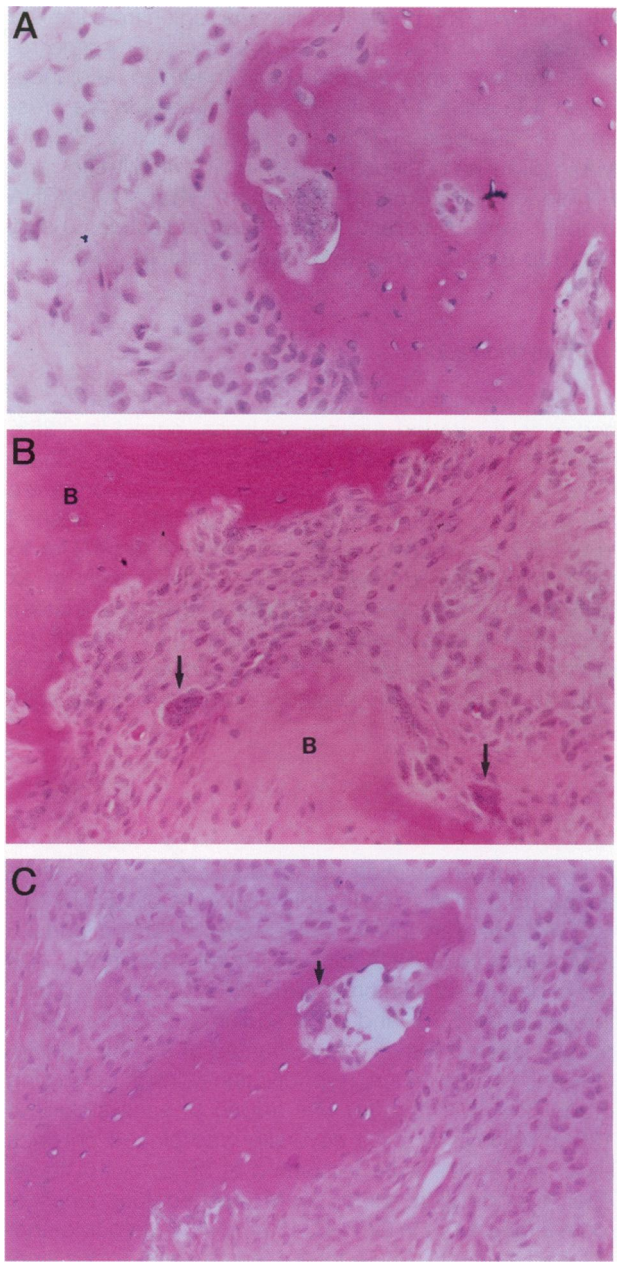

Figure 2. *In situ* hybridization of tissue from a patient with rheumatoid arthritis (RA), identifying cells expressing TRAP mRNA. **A:** Black grains designating a MNC expressing TRAP mRNA in a bone resorption lacuna. H&E counterstain; magnification, ×100. **B:** Black grains designating MNCs (**arrows**) and mononuclear cells on the bone surface and remote from bone expressing mRNA for TRAP. B, bone. H&E counterstain; magnification, ×66. **C:** Sense control probe demonstrating lack of hybridization to MNCs (**arrow**) and mononuclear cells. H&E counterstain; magnification, ×66.

remote from bone were TRAP positive, suggesting that osteoclast precursors arise within pannus in areas remote from the bone surface (Figure 1C). *In situ* hybridization was performed with a probe for the TRAP enzyme in two cases of RA and one case of JRA and confirmed mRNA expression for TRAP in MNCs on bone, in mononuclear cells on the bone surface, and in mononuclear cells within pannus (Figure 2).

To more definitively identify osteoclasts and osteoclast precursors in these samples, *in situ* hybridization was performed using a 642-bp probe prepared from the cloned human CTR. In all cases, MNCs within resorption lacunae on the bone surface in areas of bone erosion were found to express CTR mRNA. MNCs on bone did not show hybridization to the sense control probe. This finding establishes these cells as authentic osteoclasts (Figure 3). In several foci in two cases, mononuclear cells on the bone surface were also found to express CTR

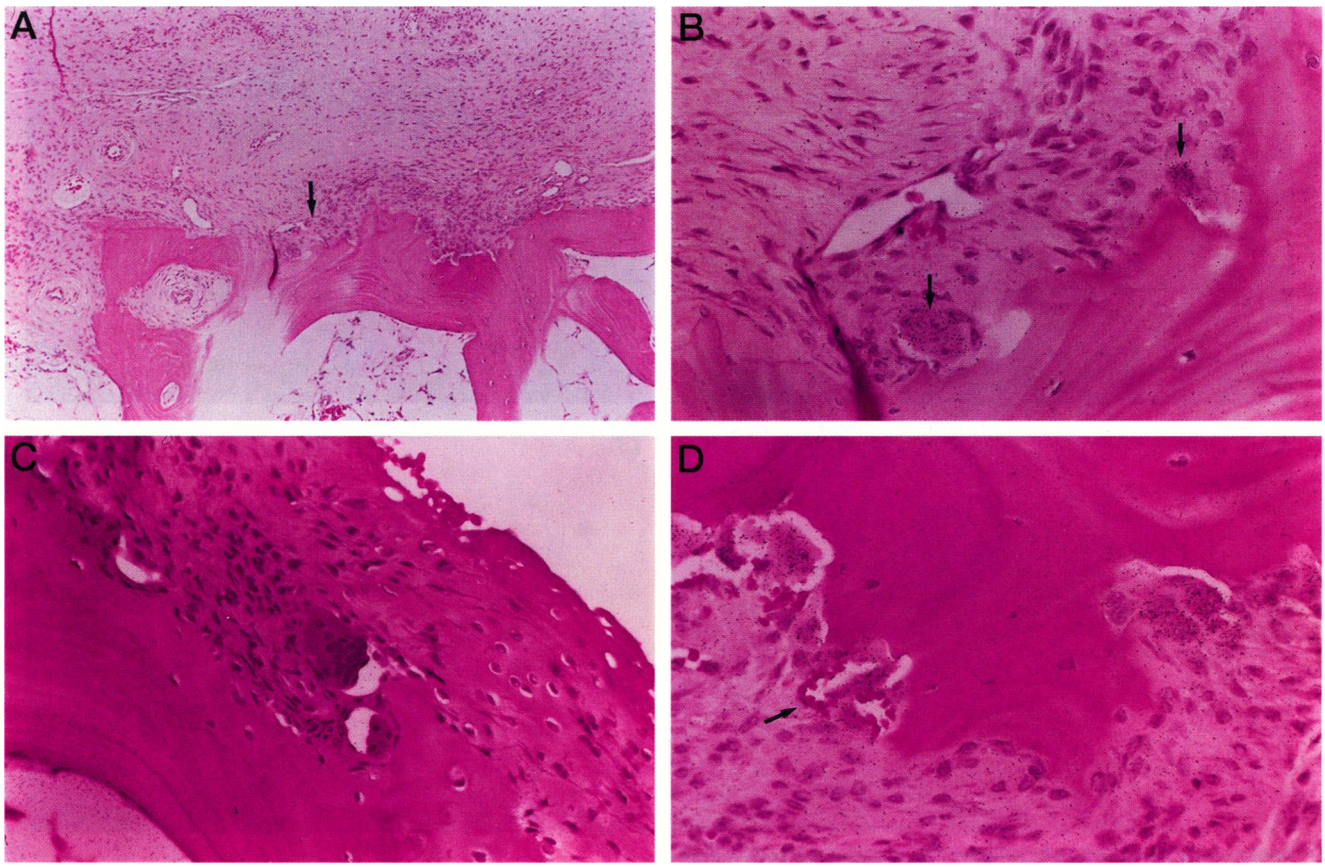

Figure 3. A: Low-power view of joint tissue from a patient with rheumatoid arthritis. The **arrow** identifies the area demonstrated in **B.** H&E counterstain; magnification, ×20. **B:** *In situ* hybridization. A high-power view of MNCs on bone surface (**arrows**) is shown. Black grains designate cells expressing mRNA for CTR. H&E counterstain; magnification, ×100. **C:** Sense control probe demonstrating lack of hybridization to MNCs on bone. H&E counterstain; magnification, ×100. **D:** High-power view of MNCs and mononuclear cells (**arrow**) on bone. Black grains designate cells on bone expressing CTR mRNA. H&E counterstain; magnification, ×100.

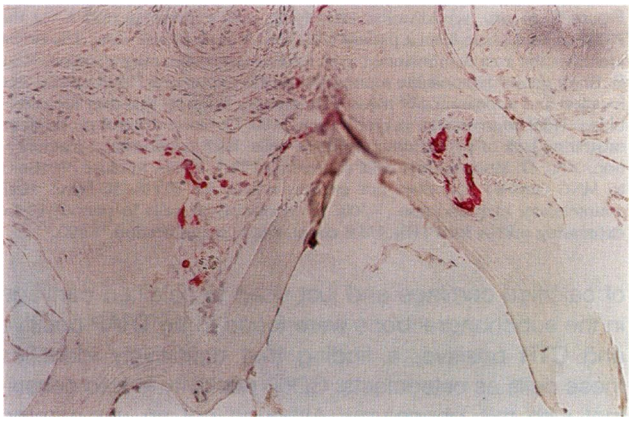

Figure 4. Immunohistochemistry demonstrating CD68-positive MNCs on bone and CD68-positive mononuclear cells on and off bone. Hematoxylin counterstain; magnification, ×50.

mRNA (Figure 3D), suggesting that these cells are osteoclast precursors that express CTR before fusion to become osteoclasts. Mononuclear cells remote from bone were uniformly CTR negative, as were MNCs in synovial tissues remote from bone.

Expression of CD68 is a valuable marker for the identification of cells of the monocyte/macrophage lineage and is also expressed by authentic osteoclasts. Synovial samples, including regions from the bone-pannus interface, were stained for CD68 by immunohistochemistry. MNCs on bone were uniformly CD68 positive, consistent with their identity as osteoclasts. In addition, numerous mononuclear cells on the bone surface in areas of osteoclastic bone resorption were also CD68 positive, and pannus tissue contained scattered to frequent CD68-positive cells as has been previously described,[8] including cells located at the interface of cartilage and pannus in areas of cartilage loss (Figure 4). Occasional CD68-positive cells were also localized in bone marrow and in areas of invading pannus remote from bone.

Tissue samples were also examined for the expression of receptors for PTHR. Conditions of chronic, excessive parathyroid hormone secretion leads to states of increased bone turnover and enhanced osteoclastic bone resorption, and it has been demonstrated that immobilization osteoporosis does not occur in parathyroidectomized animals.[30] PTH increases osteoclast number and activity; however, the bulk of the evidence indicates that this hormone does not act directly on osteoclasts, but rather exerts its effects indirectly via other cell types that are not of osteoclast lineage. We studied the expression of PTHR mRNA by *in situ* hybridization in tissue samples from patients with RA and JRA. MNCs in resorption lacunae on bone were uniformly PTHR negative. The predom-

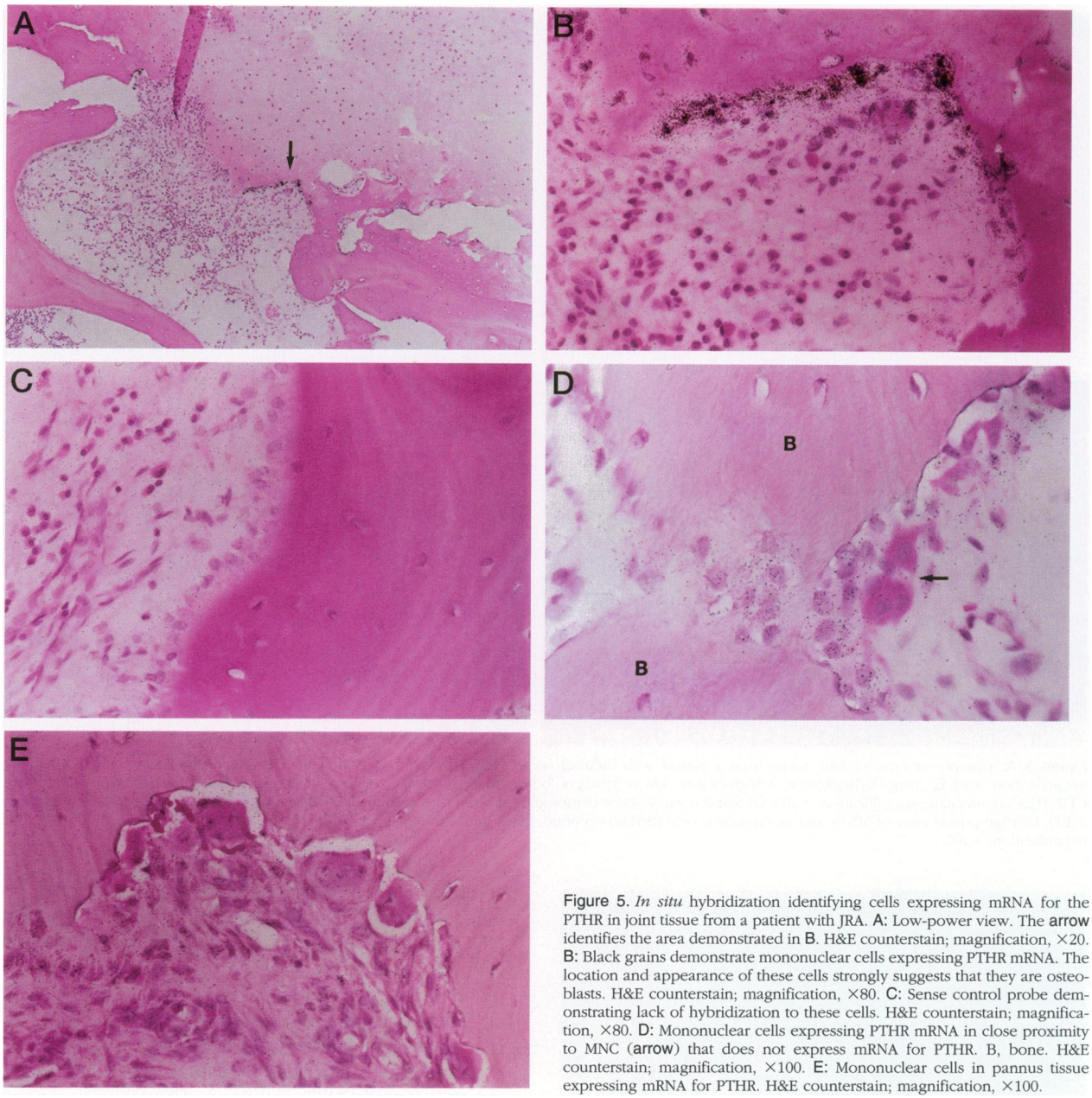

Figure 5. *In situ* hybridization identifying cells expressing mRNA for the PTHR in joint tissue from a patient with JRA. **A:** Low-power view. The arrow identifies the area demonstrated in **B**. H&E counterstain; magnification, ×20. **B:** Black grains demonstrate mononuclear cells expressing PTHR mRNA. The location and appearance of these cells strongly suggests that they are osteoblasts. H&E counterstain; magnification, ×80. **C:** Sense control probe demonstrating lack of hybridization to these cells. H&E counterstain; magnification, ×80. **D:** Mononuclear cells expressing PTHR mRNA in close proximity to MNC (arrow) that does not express mRNA for PTHR. B, bone. H&E counterstain; magnification, ×100. **E:** Mononuclear cells in pannus tissue expressing mRNA for PTHR. H&E counterstain; magnification, ×100.

inant cell types expressing PTHR mRNA were mononuclear cells located on the bone surface. These cells exhibited morphological features of osteoblasts (Figure 5, A and B). A second population of PTHR-positive mononuclear cells was located in close proximity to PTHR-negative MNCs (Figure 5D). In addition, occasional PTHR-positive mononuclear cells were present within the pannus tissue remote from MNCs and remote from bone (Figure 5E).

Discussion

In this study, we have analyzed the phenotype of the bone-resorbing cells in samples obtained from patients with RA and JRA at the time of total joint replacement arthroplasty. MNCs located in resorption lacunae in areas of calcified cartilage and just deep to calcified cartilage in the subchondral bone were found to be TRAP positive and CTR positive, a finding that definitively identifies these cells as osteoclasts. CD68 positivity is also consistent with this phenotype. Although not an unexpected finding, the role of osteoclasts in focal bone resorption has been underappreciated, and in general, therapies for RA have not been directed at specifically targeting the osteoclast.

A striking finding in this study was the number of mononuclear cells and MNCs on and off bone at the pannus-bone interface that expressed TRAP mRNA and enzymatic activity. *In vitro* studies employing bone marrow cells have established that TRAP activity is expressed in osteoclast precursors before multinucleation.[20,31] The expression of this activity roughly

coincides with the expression of the vitronectin receptor.[20,31,32] Both of these markers are expressed before expression of the CTR and thus identify cells in an intermediate stage in osteoclast differentiation. The presence of TRAP-positive mononuclear cells within and adjacent to invading pannus suggests that signals are being provided within the pannus that are capable of inducing the expression of TRAP activity. Analysis of tissue from the cartilage-pannus junction from patients with RA has shown that this tissue is an active site of synthesis and release of many cytokines, including interleukin-1α, interleukin-6, and tumor necrosis factor-α,[8,10] all of which have the capacity to directly or indirectly induce osteoclast differentiation.[33]

Data from *in vitro* explant cultures of RA synovial tissues support the concept that mononuclear cells derived from RA synovium can differentiate into cells with the potential for bone erosion. In one study, synovial fragments were digested and the resultant cell suspension was added to bone slices in the presence or absence of the rat osteoblast-like cell line UMR 106. Addition of 1,25-dihydroxyvitamin D_3, dexamethasone, and human macrophage colony-stimulating factor (M-CSF) resulted in differentiation of mononuclear cells that were TRAP, vitronectin receptor, and CTR negative into TRAP, vitronectin receptor, and CTR-positive MNCs that were capable of extensive bone resorption.[34] Furthermore, a subset of MNCs in RA synovium remote from bone have been demonstrated to express certain markers of the osteoclast phenotype, including TRAP and vitronectin receptor[17,33,35] as well as carbonic anhydrase II and vacuolar protein-ATPase,[33] although not CTR as assessed by radiolabeled binding assays using salmon calcitonin (personal observations). In another study, TRAP-positive MNCs from patients with RA have been demonstrated to form resorption pits on dentine slices in culture.[36] Expression of CTRs was not, however, evaluated.

Studies in synovium surrounding osteophytes in OA also provide evidence that cells of the monocyte/macrophage lineage within synovial tissues can differentiate into bone-resorbing cells.[37] In our own studies of PVNS,[38] we demonstrated that mononuclear cells in this synovial lesion are mainly of the monocyte/macrophage lineage. Examination of mononuclear cells clustering in areas of MNCs with phenotypic features of osteoclasts were demonstrated to express TRAP and vitronectin receptor, suggesting that they were the immediate precursors of the osteoclast-like MNCs that characterize this lesion.

In this study, we demonstrate CTR-positive mononuclear cells only on the surface of bone. Therefore, the critical signals for the expression of fully differentiated osteoclasts that express the CTR appear to derive from direct contact with mineralized tissue substrates. Findings from our previous studies suggest that bone itself may provide signals for osteoclast differentiation and activity. In these studies,[39,40] devitalized bone particles or polymeric materials were implanted into rat subcutaneous tissues, and the sequence of cellular events and phenotype of the cells that populate the local foreign body reaction were examined. When particles of polymeric materials were used, the MNCs on the particle surface exhibited features of macrophage polykaryons. In contrast, when bone particles were used, the MNCs exhibited TRAP activity and expressed CTRs consistent with their identity as authentic osteoclasts. These findings provide strong evidence that the bone itself, either as a function of its mineralized matrix or related to the presence of retained cytokines or growth factors, can provide the signals that are essential for the complete expression of the osteoclast phenotype.

Tissue sections were also examined for the presence of cells expressing the PTHR. MNCs on and off bone were uniformly negative for PTHR mRNA. These findings provide direct evidence that, in the rheumatoid lesion, osteoclasts do not express receptors for PTH and thus are not directly regulated by this hormone. There is controversy concerning whether osteoclasts respond directly to PTH. PTH binding sites have been reported on avian osteoclasts in culture,[41,42] and stimulation by PTH of osteoclast-like cell formation from precursor cells has also been demonstrated.[43] However, the bulk of evidence suggests that the up-regulation of osteoclastic bone resorption by PTH is mediated indirectly via effects on cells of osteoblast lineage.[20] This is consistent with our observations, given that the cells on bone that expressed PTHR exhibited morphological features of osteoblasts. Our observations do not exclude the possibility that PTHR is expressed on MNCs at low levels that would not be detectable by *in situ* hybridization. However, analysis of a variety of other human bone tissues, including samples of bone from patients with Paget's disease, also demonstrate that the osteoclasts are devoid of PTH mRNA (unpublished observations).

It is of interest that many of the cells expressing PTHR appeared to cluster adjacent to sites of erosions containing CTR-positive osteoclasts, suggesting that these cells are also osteoblasts. A mounting body of evidence supports a role for an interplay between osteoblast lineage cells and osteoclasts.[20] When stimulated by PTH, human osteoblasts in culture have been demonstrated to secrete matrix metalloproteinases, such as collagenase, which may help prepare bone surfaces for osteoclastic action by degrading the surface osteoid layer and exposing the mineralized matrix.[44] Alternatively, there are data suggesting that early hematopoietic precursors of osteoclasts are PTH responsive,[45,46] and thus, we cannot exclude the possibility that the cells clustering adjacent to the MNCs in resorption lacunae are PTHR-positive precursors of osteoclasts.

The exact role of PTH in the various forms of bone disease in patients with RA is unclear. However, it has been proposed that PTH might contribute to periarticular bone loss, as plasma levels of PTH and calcitriol were predictive of bone loss in patients with early RA in one study.[47] *In vivo* and *in vitro* studies have suggested that PTH can amplify the inductive effects of interleukin-1 and other mediators on bone resorption.[48] In addition, Crisp et al[49] have demonstrated exacerbation of bone disease in one RA patient with coexistent primary hyperparathyroidism. Of interest, parathyroidectomy resulted in improvement in the disease.

In conclusion, our studies provide strong evidence that a component of the focal bone erosions associated with the articular bone lesions in patients with RA and JRA is attributed to cells expressing definitive features of osteoclasts, including the expression of CTR. These findings are of considerable clinical interest as they indicate that therapeutic interventions that specifically inhibit osteoclast-mediated bone resorption represent a rational approach for pharmacological intervention.

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